and quantitatively different in vesicle-rich and vesicle-poor synapses and depends on the spatial localisation of the synapse and their number of neighbors, respectively. This variation could be the basis for specific information-processing circuits in the hippocampus.

3554-Pos

Simultaneous Optical-Electrical Measurement of the Delay between formation of the Fusion Pore and Proton Equilibration in Exocytosis of Single Vesicles

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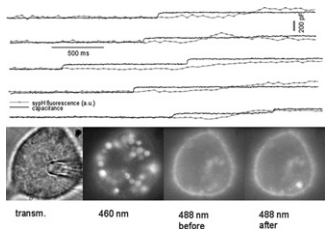
Optical detetction of neutralization of pH in granules or vesicles is often used to define exocytotic events. However, combined measurents of ensemble capacitance and pH-dependent vesicular fluorescence changes have suggested that the movement of protons only becomes possible after fusion pore expansion with a mean delay of $>$ 300 ms (1). To enhance the temporal resolution of such measurements, we have combined capacitance recordings of single vesicle fusion in RBL cells transfected with synapthpHluorin as a reporter of vesicular pH. To monitor cell capacitance steps due to exocytosis of single granules in whole cell patch-clamp mode, we used the piecewise linear technique. Internal solution contained 10 μM free Ca2+ and 300 μM GTPγS. Before establishing whole cell recordings, punctate fluorescence signals could be detected with excitation at 460 nm, while during perfusion with internal solution and excitation at 480 nm, punctate fluorescence signals gradually appeared at corresponding sites. Fluorescence increases clearly lagged capacitance steps by several 100 ms- seconds, supporting the idea that pH equilibration through the fusion pore is delayed.



(1) Barg et al.: Neuron, 33, 287-299, 2002.

Intracellular Channels

3555-Pos

Functional Properties of SR Cl^- and K^+ Channels during Postnatal Development of Cardiac Muscle

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In adult (AD) heart, the sarcoplasmic reticulum (SR) contains Cl^- & K^+ channels presumably involved in controlling RyR-mediated SR Ca^{2+} release. These channels provide a countercurrent mechanism that attenuates the drop in Ca^{2+} driving force across the SR membrane, thereby preventing early termination of Ca^{2+} release. We showed that in newborn (NB), Ca^{2+} sparks occur with similar frequency than in AD but have shorter duration and smaller amplitude, implying an early termination of Ca^{2+} release. Although the functional properties of SR Cl^- & K^+ channels have been thoroughly described in AD, little is known about their presence and their role in NB. Consequently, we first tested the hypothesis that the early termination of Ca^{2+} release in NB coincides with absence/low density of SR Cl^- & K^+ channels at this stage. To this end, the heavy microsomal fraction was obtained from 5-days-old NB and AD rat hearts and SR Cl^- & K^+ channels were reconstituted into artificial planar lipid bilayers. Our results indicate that Cl^- & K^+ channels can be reconstituted from NB heavy SR microsomes with a similar success rate (number of SR channel incorporations / total number of bilayers) than in AD (~0.2 for Cl^- channels & ~0.1 for K^+ channels). Thus, an alternative mechanism would imply that in NB, smaller counterion fluxes result from different functional properties of SR Cl^- & K^+ channels. This assumption was tested by measuring their unitary conductance, open probability, and voltage dependence. The results in NB channels revealed no significant differences in any of these parameters in comparison to AD. Thus, we concluded that SR Cl^- & K^+ channels do not contribute to the developmental changes of Ca^{2+} release in NB cardiomyocytes. Supported by AHA-0655656Z to RMA.

3556-Pos

Role of TRIC-A Channel in Circulatory Function

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TRIC (trimeric intracellular cation) channels in the sarco-/endoplasmic reticulum likely act as counter-ion channels that conduct monovalent cations in a synchronized manner with release of stored Ca^{2+}. TRIC channel subtypes display differential expression patterns as TRIC-A is predominantly expressed in excitable tissues, including brain and muscle, and TRIC-B is present throughout many tissues. TRIC-A knockout mice are viable and fertile, while TRIC-B knockout mice exhibit neonatal lethality due to respiratory failure (Yamazaki et al., Development 2009), and double-knockout mice lacking both subtypes show embryonic cardiac failure (Yazawa et al., Nature, 2007). To resolve the physiological role of TRIC-A, we are currently focusing on abnormal circulatory function in TRIC-A-knockout mice during young adulthood. These mutant mice showed significant hypertension and bradycardia. Autonomic blocking agents (co-application of atropine and metoprolol) greatly improved the bradycardic condition without affecting hypertension in the mutant mice. This observation suggests that a hyperactive baroreceptor reflex leads to development of the bradycardic condition in the mutant mice. Blockers for vasoactive humoral factors, such as angiotensin, endothelin and vasopressin, did not significantly improve hypertension in the mutant mice, suggesting normal blood-vasopressor levels. Importantly, isometric tension measurements indicated that contractility is markedly impaired in aortic ring preparations from the mutant mice, and that acetylcholine-induced relaxation is hypersensitive in mutant mesenteric artery. Our results suggest a vital role for TRIC-A channels in the physiological regulation of vessel tonus by vascular smooth muscle and endothelial cells. To further examine the pathogenesis of hypertension at the molecular level, we plan to examine TRIC-A expression and agonist-evoked Ca^{2+} transients in smooth muscle and endothelial cells from TRIC-A-knockout and wild-type mice.

3557-Pos

Tic110 a Channel-Forming Protein at the Inner Envelope of Chloroplasts Electrophysiology and Regulation

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Tic110 has been proposed to be a channel-forming protein at the inner envelope of chloroplasts whose function is essential for the import of proteins synthesized in the cytosol. Sequence features and topology determination experiments presently summarized suggest that Tic110 consists of six transmembrane helices. Its topology has been mapped by limited proteolysis experiments in combination with mass spectrometric determinations and cysteine modification analysis. Two hydrophobic transmembrane helices located in the N terminus serve as a signal for the localization of the protein to the membrane as shown previously. The other amphipathic transmembrane helices are located in the region composed of residues 92-959 in the pea sequence. This results in two regions in the intermembrane space localized to form supercomplexes with the TOC machinery and to receive the transit peptide of preproteins. A large region also resides in the stroma for interaction with proteins such as molecular chaperones. In addition to characterizing the topology of Tic110, we show that Ca2+ has a dramatic effect on channel activity in vitro and that the protein has a redox-active disulfide with the potential to interact with stromal thioredoxin.

3558-Pos

Luminal Ca^{2+} is a Major Sensitiser of Two-Pore Channels to NAADP

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It has been suggested that two-pore domain channels (TPCs) are the NAADP receptors responsible for NAADP-mediated Ca^{2+}-release from lysosome-related stores yet there is evidence that NAADP could also regulate RyR channels. We have therefore compared the effects of NAADP on native RyR1, RyR2 and purified human TPC2, reconstituted into artificial membranes under identical experimental conditions. Similar to RyR channels, we find that TPC2 behaves as an ion-channel permeable to both monovalent (300 ± 14pS; symmetrical 210 mM K^+; SD; n=3) and divalent cations (15 ± 2pS; 10μM *cis*/50 mM *trans* Ca^{2+};SD; n=5) with no evidence for anion permeability (in a 210mM *trans*: 510mM *cis* KCl gradient, the reversal potential coincides with the calculated value for a channel ideally selective for cations (E_{rev}=-23mV)). Addition of *trans* NAADP had no effect on TPC2, but *cis* ap-